CYANIDE AND THIOCYANATE-BASED BIOSYNTHESIS IN TROPICAL MARINE SPONGES

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The sponge *Axinyssa* n.sp. incorporates both sodium [^{14}C] cyanide and sodium [^{14}C] thiocyanate into 2-thiocyanatoneopupukeanane as well as into 9-isothiocyanatopupukeanane, however these 2 precursors were not incorporated into 9-isocyanopupukeanane. The specificity of incorporation into the thiocyanate carbon was confirmed by chemical degradation. *Stylotella aurantium* incorporates sodium [^{14}C] cyanide and sodium [^{14}C] thiocyanate into the dichloroimine functionality of the stylotellanes A and B, as well as into farnesyl isothiocyanate. The specificity of incorporation into the dichloroimine carbon atom was confirmed by chemical degradation. These experiments represent the first detailed study of the biosynthetic origin of organic thiocyanates and dichloroimines, and extend the range of functionality known to be biosynthesised from cyanide and thiocyanate. Our results raise the interesting question of the interconversion of inorganic cyanide and thiocyanate and/or the interconversion of the resulting organic metabolites in marine sponges. An isothiocyanate-isocyanide conversion was demonstrated in *Amphimedon terpenensis* by incorporation of a ^{14}C-labelled sample of diisothiocyanatoadociane into diisocyanoadociane. □ *Porifera, Amphimedon terpenensis, Axinyssa, Stylotella aurantium, biosynthesis, cyanide, dichloroimines, isocyanides, isothiocyanates, secondary metabolites, terpenes, thiocyanates.*

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Marine sponges of the order Axinellida, Halichondrida and Haplosclerida often contain bioactive terpenes with isocyanide, isothiocyanate and formamide functionality; the rarer isocyanate and thiocyanate substituents are also known (Scheuer, 1992; Chang & Scheuer, 1993; Garson et al., 1998). These unique metabolites have been novel targets for study with ^{14}C- and ^{13}C-labelled precursors to determine the biosynthetic origin of the non-terpenoid carbon atom (Garson, 1989; Chang & Scheuer, 1990; Garson, 1993; Garson et al., 1999). Work by our research group on the sponge *Amphimedon terpenensis* has shown that marine isocyanides such as diisocyanoadociane (Fig. 1A) are derived by functionalisation of a terpene precursor with inorganic cyanide (Garson, 1986; Fookes et al., 1988). Karuso & Scheuer (1989) subsequently showed that both diterpene (eg. Fig. 1B) and sesquiterpene (eg. Fig. 1C) isocyanides are cyanide-derived, and further demonstrated the intact incorporation of the N_1-C_1 unit. Our recent work with *Acanthella cavernosa* has shown the utilisation of cyanide for the biosynthesis of both a sesquiterpene isocyanide (Fig. 1D) and an isothiocyanate (Fig. 1E) in this axinellid sponge.

Furthermore inorganic thiocyanate was shown also to be a precursor to both the isocyanide and the isothiocyanate metabolites in this sponge. From these experimental results a biosynthetic link was inferred between the two inorganic precursors or between the two metabolite types (Dumdei et al., 1997).

The origin of the thiocyanato group has been the subject of much biosynthetic speculation (Garson, 1993). Pham et al. (1991) suggested the cyanation of a thiol, which appears to be a reasonable pathway to the amino acid-derived psammaplin thiocyanate (Jimenez & Crews, 1991). In contrast, in those sponges in which thiocyanates co-occur with isocyanides or with isothiocyanates, the involvement of the ambident thiocyanate ion has been invoked (He et al., 1989; 1992; Walker & Butler, 1996). The dichloroimine (= carbonimidic dichloride) moiety represents a rare example of a functional group containing both nitrogen and carbon which has previously been found in terpene metabolites of the Indo-Pacific sponge *Pseudaxinyssa pitys* (Wratten & Faulkner, 1977; 1978a; 1978b). The coocurrence of an isothiocyanate together with dichloroimines in *P. pitys* suggested to us the involvement of
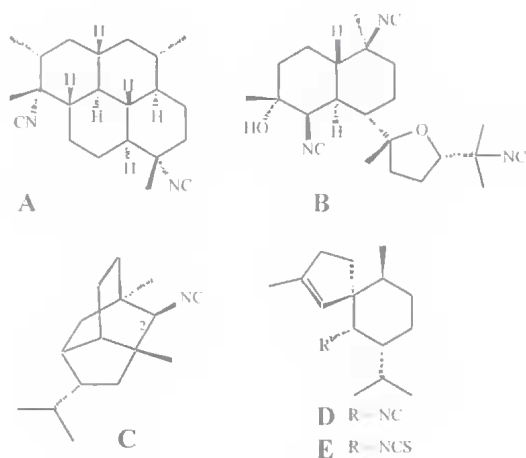
FIG. 1. Structures of isocyanide and isothiocyanate metabolites investigated in biosynthetic experiments. A, diisocyanoadociane. B, kalihinol F. C, 2-isocyanopupukeanane. D, axisonitrile-3. E, axisothiocyanate-3.

cyanide/thiocyanate in the biosynthesis of the dichloroimine group.

In this paper we present the results of biosynthetic experiments with the sponge *Axinyssa* n. sp. which provide evidence for a cyanide/ thiocyanate origin of the thiocyanato functionality. We test the possibility using *Stylotella aurantium* that dichloroimine metabolites are biosynthesised from farnesyl pyrophosphate using cyanide or thiocyanate to supply the N_1-C_1 moiety. The role of inorganic thiocyanate and of an organic isothiocyanate in diisocyanoadociane biosynthesis are also explored.

MATERIALS AND METHODS

Abbreviations. GC-MS, gas chromatography- mass spectrometry; TLC, thin layer chromatography; NMR, nuclear magnetic resonance; HPLC, high performance liquid chromatography.

Chemicals and biochemicals. Solvents used in the extraction of compounds from sponge samples were glass distilled. All radioactive precursors were purchased from Sigma Chemical Co. (St Louis, MO).

Biological materials. Samples of *Axinyssa* n. sp. (Halichondrida: Halichondriidae), *Stylotella aurantium* (Halichondrida: Halichondriidae), Kelly-Borges & Bergquist, 1988, and *Amphimedon terpenensis* (Haplosclerida: Niphatidae) Fromont, 1993, were collected using SCUBA at Coral Gardens, Experimental Gardens or Coral Spawning dive sites (12-16m depth), Heron Island (23°27'S, 151°55'E) or at North Point (12-16m depth), Lizard Island (14°39'S, 145°27'E) on the Great Barrier Reef, Australia under permit numbers G96/050, G97/097, G98/037 and G98/227 issued jointly by the Great Barrier Reef Marine Park Authority and the Queensland National Parks and Wildlife Service. Sponge samples used in biosynthetic experiments were maintained in running seawater at ambient temperature and light conditions prior to use. Voucher specimens of the sponges *Axinyssa* n. sp., (accession number QMG312575), *Stylotella aurantium* (QMG307133) and *Amphimedon terpenensis*, (AMZ4978; QMG314228), are held at the Queensland Museum (QM), Brisbane or the Australian Museum (AM), Sydney.

Isolation of metabolites. 1) Axinyssa n. sp. An organic extract was prepared from frozen sponge (49.6g wet wt) and further purified by normal phase flash chromatography (gradient elution with hexanes/EtOAc) and normal phase HPLC using 0.25% EtOAc in hexanes to give (-)-9-isocyanopupukeanane (Fig. 2A; 107.6mg), (-)-9-isothiocyanatopupukeanane (Fig. 2B; 3.5mg), and (-)-2-thiocyanatoneopupukeanane (Fig. 2C; 31.4mg) together with smaller amounts of other isocyanides and isothiocyanates as described by Simpson et al. (1997b). 2) *Stylotella aurantium.* An organic extract was prepared from frozen sponge (204g wet wt) and further purified by normal phase flash chromatography (gradient elution with hexanes/EtOAc) and by normal phase HPLC using 0.2% EtOAc in hexanes to give stylotellane A (Fig. 2E; 9mg), stylotellane B (Fig. 2F; 75.6mg), and farnesyl isothiocyanate (Fig. 2G; 2mg) as described by Simpson et al. (1997a). 3) *Amphimedon terpenensis.* Diisocyanoadociane (Fig. 1A; 16mg) was isolated from frozen sponge (25g wet wt) as described by Fookes et al. (1988).

Biosynthetic experiments. 1) Pieces of *Axinyssa* n. sp. (approx. 80g wet wt) were placed in an aquarium containing 200ml aerated seawater at ambient temperature (20-23°C). Sodium [^{14}C] cyanide (100μCi) or sodium [^{14}C] thiocyanate (25μCi) was added and the sponge allowed to assimilate radioactivity for 12hr. The sponge was kept in running seawater in a 10 litre aquarium at ambient temperature for 16 days, then frozen for subsequent radiochemical analysis. Metabolites were purified according to the above protocol. The radioactivity content was monitored at each stage of the purification sequence, and terpenes were subjected to repeated HPLC until the specific activity was constant. 2) *Stylotella aurantium* (24g

wet wt) was placed in an aquarium containing 200ml aerated seawater at ambient temperature (20-23°C). Sodium [^{11}C] cyanide (50μCi) was added and the sponge allowed to assimilate radioactivity for 12hr overnight. The sponge was kept in running seawater in a 10L aquarium at ambient temperature for 9 days, then frozen for subsequent radiochemical analysis. Metabolites were purified according to the above protocol. The radioactivity content was monitored at each stage of the purification sequence. A sodium [^{14}C] thiocyanate (13μCi, 9 days incorporation) experiment, used a 12g piece of sponge. 3) *Amphimedon terpenensis* (26g wet wt) was placed in an aquarium containing 400mL aerated

FIG. 2. Structures of terpenes isolated from *Axinyssa*.sp., *Stylotella aurantium* and degradation products. A, 9-isocyanopupukeanane B 9-isothiocyanatopupukeanane. C, 2-thiocyanatoneopupukeanane. D thiol from 2-thiocyanatoneopupukeanane. E, stylotellane A, F stylotellane B. G, farnesyl isothiocyanate. G, methyl carbamate from stylotellane B. J, amine from stylotellane B.

seawater at ambient temperature (20-23°C) [^{14}C]-Diisothiocyanatoadociane (11μCi) was added and the sponge allowed to assimilate radioactivity for 12hrs overnight. The sponge was kept in running seawater in a 20L aquarium at ambient temperature for 19 days, then frozen for subsequent radiochemical analysis. Metabolites were purified according to the above protocol. The radioactivity content was monitored at each stage of the purification sequence. A sodium [^{14}C] thiocyanate (50μCi; 19 days incorporation) experiment used a 45g piece of sponge.

Procedures used in the synthesis of [^{14}C]-diisothiocyanatoadociane will be described elsewhere (Simpson & Garson, in preparation).

RESULTS

1) *Axinyssa* n.sp. collected at Heron I. contained sesquiterpene metabolites by GC-MS, TLC and NMR; the hexane-solubles were processed as described in Simpson et al. (1997b) to give the 9-pupukeanane isocyanide/isothiocyanate pair (Fig. 2A,B) and 2-thiocyanatoneopupukeanane (Fig. 2C). The GC-MS profile of the sesquiterpene fraction showed a number of other peaks including isocyanides and isothiocyanates. Light and electron microscopic inspection of *Axinyssa* n.sp. revealed the presence of microbial symbionts. The outer layers of sponge tissue were rich in cyanobacteria of a type morphologically similar to *Aphanocapsa feldmanni* while the
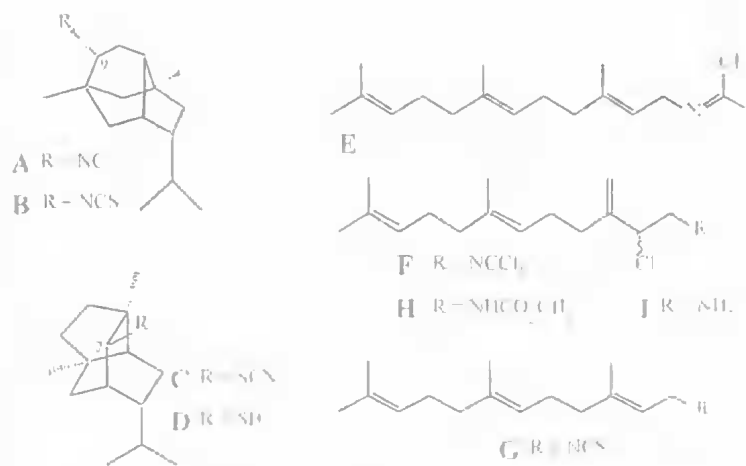
inner tissue contained high populations of diverse bacterial cell types in addition to sponge cells. An Archaea-like symbiont with a membrane-bound nucleoid was found (see Fuerst et al., this volume; Fuerst et al., 1998).

25μCi Sodium [^{14}C] thiocyanate was supplied to a specimen of *Axinyssa* n.sp. maintained in a small aquarium (Dunde et al., 1997; Simpson & Garson, 1998). After 16 days aquarium incubation, the sponge sample was frozen and 2-thiocyanatoneopupukeanane (Fig. 2C) was isolated and rigorously purified by HPLC to constant specific radioactivity. The thiocyanate (Fig. 2C) was significantly radioactive, as shown in Table 1 consistent with the use of thiocyanate for the biosynthesis of the thiocyanato group as shown in Fig. 3. To test the specificity of incorporation, 2-thiocyanatoneopupukeanane (Fig. 2C) was degraded to the thiol (Fig. 2D) using LiAlH$_4$. The thiol product was not radioactive (Table 2) therefore the [^{14}C] label was exclusively associated with the thiocyanato carbon. Incorporation of sodium [^{14}C] cyanide into a second piece of sponge also gave radioactive 2-thiocyanatoneopupukeanane (Table 1). Degradation resulted in unlabelled thiol product (Table 2) indicating the label was again exclusively associated with the thiocyanato moiety.

Our experiments also allowed us to monitor isocyanide/isothiocyanate biosynthesis in this sponge. When the isocyanide/isothiocyanate pair

TABLE 1. Molar specific activities of *Axinyssa* n. sp. metabolites. a, published incorporation values were not percentage values (Simpson & Garson, 1998); b, incorporation of 25μCi; c, <10^{-2} %; d, incorporation of 100μCi.

Compound (Fig. no.)	Precursor	Molar specific activity (mCi/mMole)	Incorporation[a] (%)
2A	Na[^{14}C]SCN[b]	0.004	c
2B	Na[^{14}C]SCN[b]	2.630	0.08
2C	Na[^{14}C]SCN[b]	0.150	0.02
2A	Na[^{14}C]CN[d]	0.014	c
2B	Na[^{14}C]CN[d]	13.900	0.3
2C	Na[^{14}C]CN[d]	1.230	0.2

TABLE 2. Molar specific activities of *Axinyssa* n. sp. degradation products.

Compound (Fig. no.)	Precursor	Molar specific activity (mCi/mMole)	Radioactivity (%)
2C	Na[^{14}C]SCN	0.150	100.0
2D	Na[^{14}C]SCN	<0.001	0.3
2C	Na[^{14}C]CN	1.230	100.0
2D	Na[^{14}C]CN	0.001	0.1

(Fig. 2A,B) were isolated, the isothiocyanate samples were radioactive (>150,000dpm/mg), whereas the isocyanide samples from both thiocyanate and cyanide feedings were not significantly labelled (<100dpm/mg). The specificity of labelling of 9-isothiocyanato-pupukeanane is currently under investigation.

2) Extracts of the sponge *Stylotella aurantium* weakly inhibited the growth of a P388 mouse leukaemia cell line and contained terpenes by TLC and NMR. The DCM-soluble components of the extract were processed as described in Simpson et al. (1997a) to give the stylotellanes A and B (Fig. 2E,F), together with farnesyl isothiocyanate (Fig. 2G). Light microscopic inspection of sponge tissue revealed the absence of microbial symbionts other than bacteria.

50μCi Sodium [^{14}C] cyanide was supplied to a specimen of *S. aurantium* according to our established protocols (Dumdei et al., 1997; Simpson et al., 1997a). After 9 days aquarium incubation, the sponge sample was frozen and stylotellanes A and B were isolated and rigorously purified by HPLC to constant specific radioactivity. The samples of stylotellanes A and B (Fig. 2E,F) were significantly radioactive, as
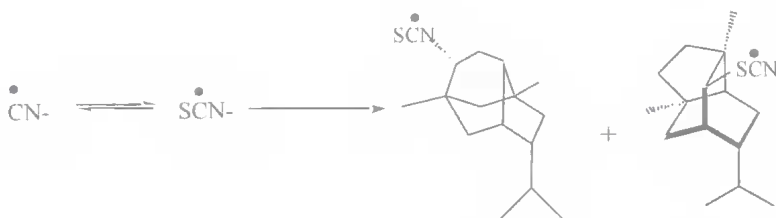
shown in Table 3, consistent with the use of cyanide for the biosynthesis of the dichloroimine group (Fig. 4, route notation 'a'). The percentage incorporation levels measured were low as a result of loss of volatile metabolites during the purification process combined with the chemical instability of the dichloroimine group. To test the specificity of incorporation, stylotellane B was degraded to the methyl carbamate (Fig. 2H) and the amine (Fig. 2I) using 0.1N phosphoric acid in 95% methanol. The carbamate product was radioactive, whereas the amine was devoid of radioactivity (Table 4), therefore the [^{14}C] label was exclusively associated with the imine carbon. Incorporation of sodium [^{14}C] thiocyanate into a second piece of sponge also gave radioactive metabolites (Table 3), however there was insufficient material for chemical degradation. In each experiment, the isolated farnesyl isothiocyanate (Fig. 2G) was also radioactive.

3) 50μCi Sodium [^{14}C] thiocyanate was then supplied to a specimen of *A. terpenensis* according to our established protocols (Fookes et al., 1988; Dumdei et al., 1997). After 19 days aquarium incubation, the sponge sample was frozen and diisocyanoadociane isolated and rigorously purified by HPLC, then recrystallised to constant specific radioactivity. The sample was significantly radioactive consistent with the use of thiocyanate for the biosynthesis of the isocyanide group (Fig. 5). Degradative experiments are in progress to confirm the specific labelling. When a sample of diisothiocyanatoadociane, ^{14}C-labelled in both isothiocyanate groups, was provided to *A. terpenensis*, the diisocyanoadociane isolated was found to be radioactive. The specificity of labelling is under investigation.

FIG. 3. Incorporation into isothiocyanate and thiocyanate metabolites of *Axinyssa* n. sp.

TABLE 3. Molar specific activities of *S. aurantium* metabolites. a, incorporation of 50μCi; b, incorporation of 13μCi.

Compound (Fig. no.)	Precursor	Molar specific activity (mCi/mMole)	Incorporation (%)
2E	Na[^{14}C]CN[a]	1.136	0.004
2F	Na[^{14}C]CN[a]	1.472	0.033
2E	Na[^{14}C]SCN[b]	0.354	0.00034
2F	Na[^{14}C]SCN[b]	0.224	0.00056

TABLE 4. Molar specific activities of *S. aurantium* degradation products. a, after dilution with unlabelled metabolite.

Compound (Fig. no.)	Precursor	Molar specific activity (mCi/mMole)	Radioactivity (%)
2F	Na[^{14}C]CN	0.332[a]	100.0
2H	Na[^{14}C]CN	0.326	98.2
2I	Na[^{14}C]CN	0.004	1.2

DISCUSSION

Our biosynthetic experiments with *Axinyssa* n. sp. and with *S. aurantium*, together with the earlier work on *A. terpenensis* and *A. cavernosa* (Garson, 1986; Fookes et al., 1988; Dumdei et al., 1997), reveal that cyanide and thiocyanate are precursors involved in the biosynthesis of four N_1-C_1 functional groups found in marine terpenes, namely isocyanides, isothiocyanates, thiocyanates and dichloroimines.

A number of different biosynthetic pathways can be invoked to explain the origin of the thiocyanate group. Pham et al. (1991) suggested the cyanation of a terpene thiol, however this proposal does not adequately explain the co-occurrence of thiocyanates and isothiocyanates in the same sponge. The insertion of sulphur into an organic cyanide or isocyanide to give a thiocyanate is mechanistically unprecedented. Sulphur insertion into an isocyanide to give an isothiocyanate (Hagadone et al., 1984), perhaps using an enzyme functionally equivalent to rhodanese (Westley, 1973), followed by iso-merisation of the isothiocyanate to the thiocyanate is a plausible biosynthetic pathway. In the laboratory however, the isothiocyanate-thiocyanate equilibrium usually favours an isothiocyanate over a thiocyanate (Hughes, 1975). A final biosynthetic possibility is the use of an ambident thiocyanate anion to attack a terpene carbenium ion or its functional equiv-alent (He et al., 1989; 1992; Walker & Butler, 1996). Thiocyanate either reacts through the nitrogen centre generating an isothiocyanate derivative or through the sulphur generating a thiocyanate.

Results on the biosynthesis of the thiocyanate moiety are particularly informative. The incorp-oration of inorganic thiocyanate into the thiocyanate and isothiocyanate metabolites (Fig. 2B,C) of *Axinyssa* n. sp. is consistent with direct utilisation of this ambident precursor. Likewise cyanide is utilised for both thiocyanate and iso-thiocyanate biosynthesis. Our proposal is that cyanide is converted in *Axinyssa* n. sp. to thio-cyanate by the action of an enzyme similar to rhodanese (Scheivelbein et al., 1969; Westley, 1973) and then incorporated into either 9-isothiocyanatopupukeanane or 2-thiocyanato-neopupukeanane. The alternative possibility that cyanide is converted first to the isocyanide then by sulphur insertion to an isothiocyanate is less likely since cyanide was not utilised for the bio-synthesis of 9-isocyanopupukeanane in the same specimen of *Axinyssa* n. sp.

Stylotella aurantium uses both cyanide and thiocyanate as precursors for the biosynthesis of the dichloroimine and isothiocyanate groups. Fig. 4 shows two plausible biosynthetic routes to the stylotellanes A and B, one route (a) using an isonitrile intermediate and the other (b) invoking an isothiocyanate intermediate. The isolation of farnesyl isothiocyanate, but not of farnesyl isocyanide (Fig. 4A), from this sponge is con-sistent with the operation of path (b). The dichloroimine metabolites are among the most unusual of the cyanide and thiocyanate-derived terpenes. In the laboratory, isocyanide dihalides can be synthesised by addition of chlorine to isocyanides or by chlorination of isothiocyanates (Kühle et al., 1967). The biosynthetic mechanisms proposed invoke the use of a chloroperoxidase enzyme to chlorinate intermediates (Butler & Walker, 1993; Walker & Butler, 1996).

In *A. terpenensis*, our results are consistent with the use of both thiocyanate and of cyanide for isocyanide biosynthesis. Thiocyanate may perhaps be converted to cyanide by use of a peroxidase enzyme, as has been demonstrated in some bacteria (Ohkawa et al., 1971; Pollock & Goff, 1992; Westley, 1981), which is then utilised for isocyanide biosynthesis (Fig. 5).

We have previously suggested that *A. cavernosa* is able to interconvert inorganic cyanide and thiocyanate (Dumdei et al., 1997). Our current
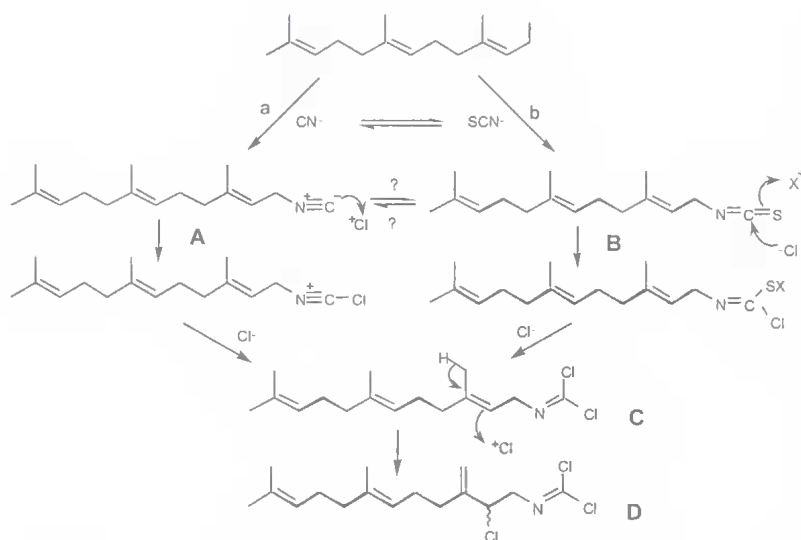
FIG. 4. Biosynthesis of dichloroimines in *Stylotella aurantium*. A, farnesyl isocyanide. B, farnesyl isothiocyanate. C, stylotellane A. D, stylotellane B.

sponge contains isothiocyanates as minor metabolites (unpublished results). When radiolabelled diisothiocyanatoadociane was supplied to samples of *A. terpenensis*, the diisocyanoadociane isolated was shown to be radioactive. Chemical degradation is currently in progress to confirm the specificity of labelling in this advanced precursor experiment.

In view of the previous successful experiments with both diterpene isocyanides (Garson, 1986; Fookes et al., 1988; Karuso & Scheuer, 1989) and sesquiterpene isonitriles (Karuso & Scheuer, 1989; Dumdei et al., 1997), it is quite extraordinary that we have not demonstrated the incorporation of cyanide into the major isocyanide component (Fig. 6) of *Axinyssa* n. sp. Likewise thiocyanate appears not to be used for isocyanide biosynthesis in this sponge, in contrast to *A. cavernosa* in which thiocyanate is used for isocyanide biosynthesis (Dumdei et al., 1997) and also in contrast to *A. terpenensis* (this paper). The lack of incorporation of thiocyanate into 9-isocyanopupukeanane (Fig. 6) suggests that either the thiocyanate to cyanide conversion is inefficient in this sponge or that the conversion of isothiocyanate into isocyanide does not occur. We are currently isolating some of the other minor isocyanide metabolites from *Axinyssa* samples labelled by thiocyanate or cyanide in order to investigate the role of cyanide and thiocyanate in isocyanide biosynthesis in this sponge. A clearer picture of the complex metabolic interrelationships in *Axinyssa* n. sp. will emerge when we test the utilisation of
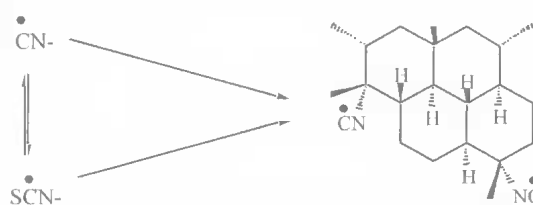
results suggest *Axinyssa* n. sp., *S. aurantium* and *A. terpenensis* are able to interconvert these 2 inorganic precursors. We have also speculated that enzymic transformations which parallel the cyanide-thiocyanate interconversion may transform organic isocyanides into isothiocyanates, or the reverse, in marine sponges (Dumdei et al., 1997). Figure 6 illustrates these suggested biosynthetic relationships for *Axinyssa* n.sp. Thiocyanate is used to make 9-isothiocyanatopupukeanane which then undergoes desulphurisation to give 9-isocyanopupukeanane; alternatively, cyanide is used for isocyanide biosynthesis, then the isocyanide is converted into the isothiocyanate by an enzyme functionally equivalent to rhodanese.

In pioneering biosynthetic experiments, Hagadone et al. (1984) inferred the precursor status of an isocyanide terpene metabolite in isothiocyanate formation in *Ciocalypta* sp. They explored the *in vivo* conversion of 2-isocyanpupukeanane into the corresponding formamide and isothiocyanate metabolites. The natural product status of formamide metabolites has however been questioned by Tada et al. (1988). A second concern with the work of Hagadone et al. (1984) is their use of the relatively insensitive ^{13}C label in conjunction with mass spectrometric detection.

Our preliminary results with *A. terpenensis* suggest that an isothiocyanate to isocyanide transformation may occur in this sponge. The

FIG. 5. Biosynthesis of diisocyanoadociane in *A. terpenensis*.

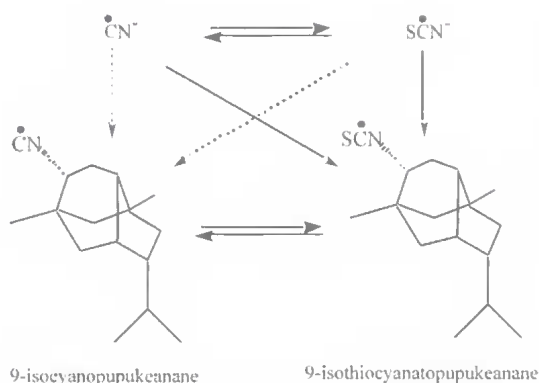9-isocyanopupukeanane   9-isothiocyanatopupukeanane

FIG. 6. Possible biosynthetic interconversions in *Axinyssa* n. sp. Solid lines indicate incorporation results or possible conversions, dotted lines indicate non-incorporation.

^{14}C-labelled isocyanide and isothiocyanate precursors by this sponge.

The origin of the cyanide or the thiocyanate used by marine sponges remains a tantalising mystery. Plants generate hydrogen cyanide by hydrolysis of cyanogenic glycosides (Seigler, 1975). Some bacteria are known to produce hydrogen cyanide (Knowles, 1976) or to convert the amino acid cysteine to thiocyanate (Voet & Voet, 1995), while methionine has been implicated in the formation of cyanide as a byproduct of ethylene biosynthesis (Pirrung, 1985). To date experiments to determine an amino acid origin for the isocyano group in diisocyanoadociane have been unsuccesful (Fookes et al., 1988).

Two sponges used in our biosynthetic experiments have interesting symbiotic profiles. *Amphimedon terpenensis* has previously been shown to contain high bacterial populations of eubacteria together with a cyanobacterial symbiont which morphologically resembles *Aphanocapsa feldmanni* (Garson et al., 1992). *Axinyssa* n.sp. contains a cyanobacterial symbiont together with numerous bacteria, in particular an archaeal-like bacteria which contains a highly unusual membrane-bound nucleoid (Fuerst, this volume; Fuerst et al., 1998). We have previously demonstrated that *A. terpenensis* isocyanides are localised in sponge cells, primarily archaeocytes and choanocytes, and infer that this is the site of synthesis of the metabolites (Garson et al., 1992). Terpene metabolites in 2 other sponges have been shown to be localised in sponge cells rather than symbiont cells (Uriz et al., 1996; Flowers et al., 1998). The range of sponges with which we are

now exploring N_1-C_1 biosynthesis provide us with additional candidates to study the cellular localisation of terpene metabolites and to explore the role of symbionts in biosynthesis.

TAXONOMIC NOTE. The sponge which we have identified as '*Amphimedon*' *terpenensis* in this paper has a chequered taxonomic history. It was first named in the literature as an *Adocia* sp. by the Roche group (Baker et al., 1976). Fromont (1993) placed the sponge within *Amphimedon* in her taxonomic studies on haplosclerid sponges of the Great Barrier Reef and proposed the species name for the large proportion of terpene metabolites. Van Soest et al. (1996) considered the skeletal characteristics were too irregular to be compatible with *Amphimedon*. Based on structural characteristics and spicule analysis, they proposed the combination *Cymbastela terpenensis*, but acknowledged however that the skeletal morphology, growth form and texture for the sponge were not typical of *Cymbastela*, as described by Hooper & Bergquist (1992). The documented secondary metabolite chemistry of *Cymbastela* spp. consists of pyrrole metabolites from a New Caledonian species (Ahond et al., 1988). Samples of *Cymbastela* sp. collected from Heron I. and Lizard I. do not have a secondary metabolite profile by NMR and GC-MS, but have been shown to contain 24-isopropyl-δ^5 sterols (Stoilov et al., 1986), whereas *A. terpenensis* contains $\delta^{5,7}$-sterols (Garson et al., 1988). A more thorough taxonomic assessment of '*A.*' *terpenensis* (and the related *C. hooperi*), including consideration of live specimen characteristics, growth form, texture and spongin content and skeletal structure is required. It is possible that a new genus is required for these species but this requires substantially more corroborative evidence than is presently available (e.g. genetic analyses). For the present we retain the taxon '*A.*' *terpenensis*, but acknowledge it does not belong with typical members of *Amphimedon* (Haplosclerida; Niphatidae).

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